bio-protocol Preprint

Not Peer Reviewed
Ask a question online

Synthesis of DSPE-PEG-GSH and DSPE-PEG-Tf

Wen Li Nitin Joshi

Updated date: Jun 23, 2021

An abbreviated version of this protocol was published in Science Advances in Dec 2020

BBB pathophysiology–independent delivery of siRNA in traumatic brain injury

DOI: 10.1126/sciadv.abd6889

Detailed protocol

Synthesis of transferrin-functionalized 1,2-distearoylsn-glycero-3-phosphoethanolamine-polyethylene glycol (DSPE-PEG)

The transferrin-functionalized DSPE-PEG was prepared according to previously described method[2,3] with some modifications:

The transferrin (Tf, from Sigma-Aldrich) was first modified with sulfhydryl (-SH) groups by reacting Tf with Traut reagent (2-iminothiolane).

1. 10 mg of Tf was dissolved in 1 ml of phosphate buffered saline (PBS, pH 8.0) containing 4 mM EDTA.
2. The Traut's Reagent (Thermo Scientific) was dissolved in the same buffer at 2mg/mL and resulted in a 14mM stock solution.
3. A 10-fold molar excess of Traut's Reagent (89 µL of the stock solution) was added into the protein solution.
4. The mixture was allowed to react for 1 hour at room temperature.
5. The thiolated Tf was separated from excess Traut's Reagent by using a Zeba Spin Desalting Column (Thermo Scientific) that has been equilibrated with PBS-EDTA buffer.

Thiolated Tf was then immediately used for conjugation with DSPE-PEG-maleimide with PEG molecular weight 3400 (DSPE-PEG-MAL) (NANOCS) via thiol Michael addition reaction.

1. The thiolated Tf was mixed with the DSPE-PEG-Mal solution at a protein-to-lipid molar ratio of 1:10. The reaction was proceeded in PBS buffer (pH 7.0 at 4 °C or room temperature for 2 hours to overnight under mild stirring.
2. Eventually, the DSPE-PEG-Tf was purified by dialysis against ultra-pure water at 4 °C overnight.

How to cite: Readers should cite both the Bio-protocol article and the original research articles where this protocol was used:

1. C. Zheng, C. Ma, E. Bai, K. Yang, R. Xu, Transferrin and cell-penetrating peptide dual-functioned liposome for targeted drug delivery to glioma. Int. J. Clin. Exp. Med. 8, 1658–1668 (2015).
2. S. J. Chiu, D. J. Liu, D. Perrotti, G. Marcucci, R. J. Lee. Efficient delivery of a Bcl-2-specific antisense oligodeoxyribonucleotide (G3139) via transferri receptor-targeted liposomes. J. Control. Release. 112, 199–207 (2006)

Synthesis of glutathione-functionalized 1,2-distearoylsn-glycero-3-phosphoethanolamine-polyethylene glycol (DSPE-PEG)

Glutathione (GSH, Sigma-Aldrich) was conjugated with DSPE-PEG-maleimide (DSPE-PEG-MAL) (NANOCS) via thiol-Michael addition reaction between t thiol group of GSH and the mal group of DSPE-PEG-mal following an established method with some modification[1-3].

1. The GSH solution (in PBS buffer, pH 7.0) was mixed with the DSPE-PEG-Mal solution at the GSH: DSPE-PEG-mal molar ratio of 2:1. They were allowed to react at 4 °C or room temperature for 2 hours to overnight under the dark and an inert nitrogen gas atmosphere.
2. Eventually, the DSPE-PEG-Tf was purified by dialysis (MWCO 1kDa) against ultra-pure water at 4 °C overnight and lyophilized into powder.

1. J. Rip, L. Chen, R. Hartman, A. van den Heuvel, A. Reijerkerk, J. van Kregten, B. van der Boom, C. Appeldoorn, M. de Boer, D. Maussang, E. C. M. d Lange, P. J. Gaillard, Glutathione PEGylated liposomes: Pharmacokinetics and delivery of cargo across the blood–brain barrier in rats *J. Drug Target.* **22**, 460–467 (2014)
2. J. N. Reginald-Opara,,D. Svirskis, S. J. O'Carroll, S. Sreebhavan, J. M. Dean, Z. Wu, Optimisation of glutathione conjugation to liposomes quantified with a validated HPLC assay. *Int. J. Pharm.*, **567**, 118451(2019).
3. H. Liu, K. D. Moynihan, Y. Zheng, G. L. Szeto, A. V. Li, B. Huang, D. S. V. Egeren, C. Park, D. J. Irvine, Structure-based programming of lymph-nod targeting in molecular vaccines. *Nature* **507**, 519–522 (2014)

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Li, W. and Joshi, N. (2021). Synthesis of DSPE-PEG-GSH and DSPE-PEG-Tf. Bio-protocol Preprint. bio-protocol.org/prep1190.

2. Li, W., Qiu, J., Li, X., Aday, S., Zhang, J., Conley, G., Xu, J., Joseph, J., Lan, H., Langer, R., Mannix, R., Karp, J. M. and Joshi, N.(2020). BBB pathophysiology–independent delivery of siRNA in traumatic brain injury. Science Advances 7(1). DOI: 10.1126/sciadv.abd6889